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STUDIES IN SEPTIC SHOCK AND OTHER PATHOPHYSIOLOGIC EFFECTS OF I--ETC(U)
AUG 75 W A ALTEMEIER DAMD17-75-C-5018

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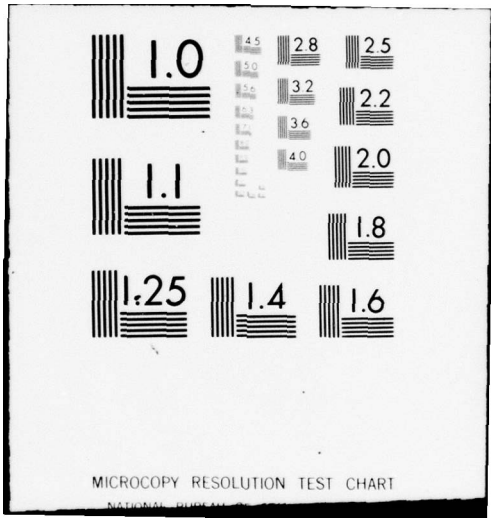
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LEVEL II

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(6) STUDIES IN SEPTIC SHOCK AND OTHER PATHOPHYSIOLOGIC EFFECTS OF INFECTION IN INJURED PATIENTS.

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by

(10) William A. Altemeier, M. D.

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The objective of this contract has been the investigation of the nature, causes, and complications of serious infections and septic shock which may occur in trauma patients. Its purpose has been to reduce the incidence, morbidity, and mortality of post-trauma infections and septic shock by improving the methods of their prevention and treatment. It represents a continuation of work previously reported under DA-49-193-MD-2531.

This report includes investigations on the following: (Over)

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20. Abstract (Continued):

1. Definition and classification of microbial and non-microbial determinants of post-trauma infections.

2. Causes of changing patterns of surgical infections.

3. Mechanisms of pathogenicity of Pseudomonas aeruginosa.

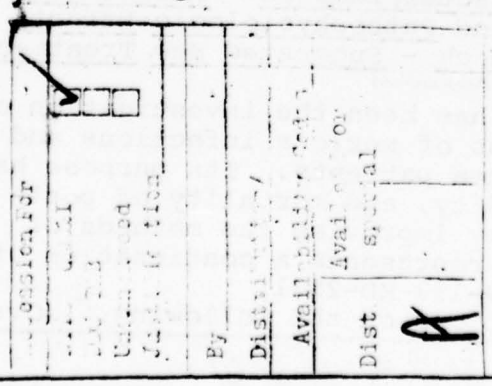
4. Evaluation of Pseufomonas vaccine and hyperimmune glovulin in ~~in~~ the prevention and control of Pseudomonas infections.

5. Factors responsible for cyclic variations in Staphylococcal antibiotic resistance.

6. Development and clinical application of indirect fluorescent antibody technics for rapid identification of Bacteroidaceae.

7. The measurement of pathophysiologic effects of clinical septic shock and comparison with those obtained in experimental animals by injections of E. coli endotoxin, the Staphylococcus aureus alpha exotoxin, or the alpha exotoxin of Cl. perfringens.

The results emphasize that septic shock is a complex syndrome in which failures of the systemic and visceral arterial tissue perfusions are integral parts. They indicate that a therapeutic advance would be possible by the infusion of a drug which would increase cardiac output, decrease peripheral resistance, and maintain a controlled mean arterial pressure to minimize secondary visceral damage and irreversibility.



Contract No. DAMD-17-75-C-5018

August 31, 1975

FINAL REPORT

STUDIES IN SEPTIC SHOCK AND OTHER PATHOPHYSIOLOGIC EFFECTS OF INFECTION IN INJURED PATIENTS

1. Date of Research Project: September 1, 1974 - August 31, 1975

2. Responsible Investigator: William A. Altemeier, M.D., Professor of Surgery and Chairman of the Department, University of Cincinnati

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4. Other Projects in which we participated and Source of Funds:

USPH - GM 15428-07: Comprehensive Clinical Study of Trauma

5. Name and Location of Institution where work was performed:

Department of Surgery, College of Medicine
University of Cincinnati
Cincinnati General Hospital

6. Head of Department where work was done: (Signature)

W. A. Altemeier, M.D.
Professor of Surgery
Chairman of the Department
University of Cincinnati

Contract No. DAMD 17-75-C-5018
FINAL REPORT

Title of Project: Studies in Septic Shock and Other Pathophysiologic Effects of Infection in Injured Patients

7. Specific Aims

7.1 Background Information and Hypothesis

The danger of serious infection has continued to be one of the greatest problems facing military or civilian patients who sustain serious injuries. After certain types of injury, infection has continued to be a significant and unsolved problem. Moreover, a number of complications of infection may develop which of themselves become life-threatening. Included among these are septic shock, renal failure, pulmonary failure, hepatic cysfunction, coagulation disorders and intravascular clotting, and severe gastrointestinal hemorrhage.

The development of post-injury infection has led to prolonged morbidity, increased mortality, excessive cost of hospitalization, unnecessary delay in wound healing, cosmetic disfigurement, loss of the injured person's capabilities to the military or industry, and increased legal liability. Moreover, the quality of life, both physical and psychological is affected and may be permanently altered.

Doctor Altemeier had been asked to go to the battle area during the Korean War by the Surgeon-General to study the cause of delayed shock which was developing in a significant number of casualties two to five days after injury. His studies there showed a causal relationship of infection to the delayed shock in many of these cases.

The significance of infection in trauma today has been recognized by the American College of Surgeons who invited Doctor Altemeier to give the Scudder Oration on that subject on October 19, 1971, and appointed a committee to define the current problems in the prevention and control of infections in wounds. Doctor Altemeier served as Chairman of this committee, and its function included the preparation of a Manual on the Control of Infections for use in Operating Rooms.

Antibiotic therapy has given us dramatic and effective methods of treating many established infections after injury. However, antibiotic therapy has not decreased the overall incidence of serious and life-threatening infections after more than a third of a century of its general use.

Of considerable interest in this regard has been the changing pattern in the types of infections from those caused by the previously more important gram-positive bacteria to the now more frequent gram-negative, anaerobic, and fungal varieties. In ou studies it has become apparent that a fourteen-fold increase in the number of cases of gram-negative sepsis has occurred during the past 16 years.

7.1 Background Information and Hypothesis (Continued)

This significant trend continued and this was reported. As we have learned how to prevent or control many types of infection, others have taken their place in the severely injured, principally those caused by the gram-negative and fungal organisms.

The gram-negative bacteria which we have found most frequently in infections complicating trauma have been: E. coli, Ps. aeruginosa, Klebsiella, Aerobacter, Proteus and Serratia marcescens. In injuries such as burns, compound fractures, and other large wounds, infections by the gram-negative bacillus Ps. aeruginosa, and more recently Proteus, Aerobacter, and Serratia have reached major proportions. The fungi most often encountered have been Candida, Aspergillus, and Sporotrichum. Antibiotic therapy has proven to be an insufficient answer to the problem of prevention or adequate treatment for Pseudomonas and fungal infections. In fact, over 80 per cent of the cases of gram-negative septicemia have occurred in patients while they were on antibiotic therapy prophylactically or for treatment for another type of infection. For this reason we have been investigating the possibility of preventing Pseudomonas infection by increasing the host-resistance of the injured patient through vaccination using a newly developed vaccine. This vaccine which was described four years ago is being prepared by the Parke Davis Company and is being used now for immunization of burn patients to evaluate its effectiveness in preventing and controlling various types of Pseudomonas infection. This work was done primarily by Doctor J. Wesley Alexander.

Our investigations were also concerned with the factors related to the change in virulence of gram-negative bacteria occurring during antibiotic therapy.

Our data continued to indicate that prophylactic antibiotic therapy has been ineffective in preventing gram-negative septicemia. Our experience has also suggested that antibiotic therapy, particularly with large and prolonged dosage, may actually contribute to the development and increasing incidence of various types of gram-negative septicemia.

The widespread use of multiple antibiotic agents has created additional problems through the development of infections produced by antibiotic-resistant bacteria emerging during therapy and sensitization of an appreciable segment of patients to be antibacterial drugs.

The emergence of Serratia marcescens septicemia and other bacterial species previously considered to be nonpathogenic as new threats in surgery has been shown by the occurrence of 64 cases of this infection studied by us at the University of Cincinnati Medical Center. This was reported in part.

7.1 Background Information and Hypothesis (Continued)

These and other considerations have established and emphasized the important hypotheses that various types of infection have continued to be a serious complication of injury, that the incidence has not been reduced by a third of a century of antibiotic therapy, that the types of infection have changed significantly, that hospital-based infections caused principally by gram-negative bacteria and associated with shock have become a serious threat to the injured patient, that infections may produce additional and little understood complications, and that septic shock and its associated pathophysiologic effects have become the most important cause of death in those complicated with septicemia.

7.2 Objectives

The objectives of this project proposed by means of clinical and laboratory research have been as follows:

1) To investigate the nature, causes, and complications of infections occurring in trauma patients.

   a) To continue studies on the determinants of infection and the changing patterns of surgical infections.

   b) To investigate the basis for the increasing incidence of gram-negative infections.

   c) To investigate mechanisms of pathogenicity of Pseudomonas aeruginosa.

   d) To investigate the significance of non-sporulating anaerobes in surgical infections.

   e) To study septic shock and the associated pathophysiologic effects of severe infections in trauma patients.

   f) To study the factors responsible for cyclic variations in antibiotic resistance of the Staphylococcus aureus recovered from surgical infections.

2) To evaluate further the effectiveness of Pseudomonas vaccine and Pseudomonas hyperimmune globulin in the prevention and control of Pseudomonas infections in burned and other seriously injured patients.

3) To investigate in burn patients the relationship between antibody formation developing from immunization with Pseudomonas vaccine and restoration of opsonic activity for Pseudomonas aeruginosa.

Continuation of Final Report on MD-5018

7.2 1) a. Study of determinants of wound infection

Previous studies done under MD-2531 have helped to clarify the determinants of infections in patients with trauma. These determinants have been classified into two important groups - microbial and nonmicrobial. Our studies of the microbial etiologic agents cultured from wound sepsis showed a large variety as illustrated in the following table.

SURGICAL INFECTIONS
ETIOLOGIC CLASSIFICATION

I. Aerobic Bacterial

A. Gram-positive cocci
1. Staphylococcus aureus
2. Streptococcus
3. Pneumococcus

B. Gram-negative cocci
1. Neisseria catarrhalis
2. Neisseria gonorrhoeae

C. Gram-negative bacillary
1. Escherichia coli
2. Aerobacter aerogenes
3. Klebsiella
4. Pseudomonas aeruginosa
5. Proteus
6. Serratia
7. Paracolon
8. Alcaligenes faecalis
9. Salmonella typhosis
10. Haemophilus influenzae

D. Gram-positive bacterial
1. Bacillus anthracis
2. Corynebacterium
3. Diphteroid
4. Bacillus species
5. Mycobacterium
a. Tuberculosis
b. Balnei

II. Microaerophilic Bacteria
Gram-positive cocci
1. Streptococcus
a. Hemolyticus
b. Non-hemolyticus

7.2 1) a. Continued:

III. Mixed Infections

A. Aerobic and Anaerobic
B. Gram-positive and Gram-negative
C. Synergistic

IV. Anaerobic Bacterial

A. Gram-positive cocci
1. Peptococcus
2. Peptostreptococcus

B. Gram-positive bacilli
1. Cl. perfringens
2. Cl. novyi
3. Cl. septicum
4. Cl. histolyticum
5. Cl. tetani

C. Gram-negative bacilli
1. Sphaerophorus necrophorus
2. Bacteroides species
3. B. melaninogenicum

V. Non-bacterial Infections

A. Fungal (wound biopsy, direct smear, and culture)
1. Candidiasis (Candida albicans)
2. Actinomycosis (Actinomyces israelii)
3. Nocardiosis (Nocardia asteroides)
4. Blastomycosis (Blastomyces dermatitides)
5. Coccidioidomycosis (Coccidioides immitis)
6. Histoplasmosis (Histoplasma capsulatum)
7. Sporotrichosis (Sporotrichum schenckii)
8. Phycomycosis (Mucor sp.)
9. Aspergillosis (Aspergillus niger)

B. Viral (Pruitt)
1. Herpesvirus
2. Poxvirus (vaccinia)
3. Varicella (Herpes Zoster virus)
4. Cytomegaloviruses
5. Mumps virus (parotitis and pancreatitis)
6. Poliovirus
7. Hepatitis virus (infectious and serum)
8. Rabies virus

Our experiments have continued to show that although microorganisms are a necessary cause of infections, they represent only part of the etiology. Other factors found to be of etiologic importance are listed in the following table.

7.2 1) a. Continued:

NON-MICROBIAL ETIOLOGIC FACTORS
OF SURGICAL INFECTIONS

1. The presence and amount of devitalized tissue within the wound
2. The presence and types of foreign bodies
3. The nature, location, and duration of the wound
4. The local and general immunity response of the individual
5. The type, time, and thoroughness of treatment
6. The general condition of the patient
7. Associated diseases
8. Iatrogenic factors

With relation to the effect of non-microbial factors as important determinants of wound sepsis, we have again focused our attention on the effects of devitalized tissue, foreign bodies, and inadequate treatment of wounds.

We used guinea pigs weighing 350 to 450 grams as the experimental animals because their marked susceptibility to Cl. welchii infection resembled that of the human being. The virulence of many strains of Cl. welchii which were obtained from a variety of sources including soil, clothing, and contaminated or infected wounds was investigated by determining the minimum lethal dose and toxigenicity for these animals. For most of the strains tested, the virulence and toxin production were either relatively low or very variable. A satisfactory strain of high virulence was obtained.

Three methods of determining the virulence of Clostridium welchii were used in previous experimental work:

1. The injection of measured amounts of a culture or its dilution intramuscularly through a fine gauged needle.

2. The injection of measured amounts of a culture or its dilution in the presence of some locally irritating substance such as calcium chloride.

3. The injection of measured amounts of a culture or its dilution into wounds containing devitalized muscle produced by mechanical means.

Experimental infections produced by the last procedure seemed to simulate more closely the type of gas gangrene which develops clinically. The following method was used to compare the virulence of Cl. welchii in healthy viable muscle with that in the presence of traumatized muscle and without contamination by sterilized dirt.

7.2 1) a. Continued:

In the first group, the skin over the lower back and posterolateral aspects of the thigh was prepared by shaving and scrubbing with soap and water for ten minutes while the guinea pigs were fastened in the prone position. The preparation was completed by the application of alcohol, ether, and tincture of iodine. Through a fine tuberculous syringe with a 25 gauge needle, 0.5 cc. of the various serial dilutions of a 4½ to 6-hour culture of Cl. welchii was injected through the skin into healthy muscle with five animals being used for each dilution.

In the second group, after the induction of drop ether anesthesia, an incision 1.0 cm. in length was made under aseptic technic through the similarly prepared skin and subcutaneous tissues of the thigh. It was developed down to and beyond the femur and the muscles on each side of the wound were crushed five times with a Kocher clamp. The wound was closed with interrupted fine black silk sutures. Injections of the serial dilutions of a culture of Cl. welchii were then made into the area of traumatized muscle through a 25 gauge needle introduced beyond the wound margins, five animals being used for each dilution.

In the third group, wounds were prepared in exactly the same manner as in the second group with the exception that 1.0 cc. of an autoclaved and finely divided mixture of soil and cinders was placed into the wound just before its inoculation with Cl. welchii.

In this way, different and measured amounts of virulent bacteria were injected into healthy muscle in one group, into areas of devitalized muscle in the second, and into areas of devitalized muscle contaminated by sterile dirt in the third. Subsequently, the characteristics and size of the wounds which developed were noted and the date of death determined in each instance. Thus, the minimum lethal dose of Cl. welchii was determined in the three groups and it was considered to be 0.5 cc. of the highest dilution which killed all the guinea pigs in 4 2/3 days under the conditions of the experiment.

Results: When serial dilutions of Cl. welchii were injected into areas of healthy muscle, the minimum lethal dose was found to be 0.5 cc. of a 10^{-2} dilution. However, when serial dilutions were injected into wounds containing crushed devitalized muscle, the minimum lethal dose was found to be 0.5 cc. of a 10^{-5} dilution. It took 1,000 times less bacteria to produce consistently a fatal gas gangrene in the presence of devitalized muscle than it did when injections were made into healthy muscle. In the third group, serial dilutions were injected into wounds containing both devitalized muscle and pulverized sterile dirt, the minimum lethal dose was 0.5 cc. of 10^{-8} dilution.

7.2 1) a. Continued:

Thus, in two experiments done on different days, it took one million times less bacteria to produce a fatal gas gangrene in guinea pigs in the presence of devitalized muscle and dirt than it did when injections were made directly into healthy muscle. The lesions of gas gangrene which developed in the animals injected with a lethal dose of Cl. welchii were very extensive and severe.

While some of the factors precipitating this infection are still unknown, these experimental results clearly illustrate the effect that devitalized muscle and dirt as non-microbial factors have in enhancing the virulence of Cl. welchii in wounds of violence, thereby acting as determinants favoring the development of gas gangrene.

Our continuing studies of wound sepsis have emphasized the changing patterns of infection which have recently occurred and which are continuing to occur. These include the following:

a. The increasing incidence of gram-negative bacillary infection.

b) The increasing incidence of gram-negative and gram-positive bacteria of low virulence as determined in previous experiments.

c) Increasing awareness of the incidence of anaerobic microorganisms as etiologic agents, including the Peptostreptococcus, Bacteroides, and Clostridia.

d) The demonstration of L-forms and other atypical bacterial forms in primary and metastatic areas of surgical infections, particularly during active antibiotic treatment of seriously injured patients.

7.3 Work in Progress

b. Investigation of the increasing incidence of gram-negative infections.

Studies of the Changing Patterns of Surgical Infections

The continuing record of the bacteria associated with surgical infections seen on the surgical services of the University of Cincinnati has been maintained and studied in the Surgical Research Bacteriology Laboratory beginning in January, 1942. These data have shown a number of interesting trends in infections occurring throughout this period. Our data showed that between 1942 and January, 1956, the majority of surgical infections, both postoperative, wound, and hospital-acquired, were caused by gram-positive bacteria, particularly the hemolytic Staphylococcus aureus. Since 1956, there has been increasing evidence that significant changes in the types of infection have occurred. Some of the more interesting changes which we have studied included the following:

1. The progressive increase in the number of Gram-negative bacterial infections.

2. The rapid and progressive increase in the number and variety of hospital-based surgical infections.

3. The development of an expanding number of infections caused by bacteria of relatively low virulence, particularly of the Gram-negative bacillary group.

4. The recognition of a growing number of clinical conditions associated with the presence and growth of bacterial "L" forms and other atypical bacterial forms.

Our studies have indicated that gram-negative bacterial infections have become a serious threat in modern surgical practice during the past twenty years. Between 1957 and 1970, there has been a fourteen-fold increase in the number of Gram-negative infections studied.

c. Pathogenicity Studies of Pseudomonas aeruginosa

In addition to the published abstract attached, the previously reported animal burn model continues to be used in an effort to demonstrate a toxic event in fatal Ps. aeruginosa burn wound sepsis. Gentamicin treatment experiments suggest that death of burned mice infected with our Ps. aeruginosa is due to a lethal event which cannot be abated once bacterial number reach 10^8/gram of burned skin even though significant reduction in viable counts can be achieved.

Immunization of burn patients with polyvalent Pseudomonas antigen (Pseudogen^R) has continued during the past year. There have been no deaths from Pseudomonas infection in immunized patients during the last two years.

7.3 Continued:

The project which attempted to immobilize Pseudomonas antigen to Sepharose has been successful. By removing lipidA from the endotoxin molecule, the glycolypopolysaccharide can be attached, and we have been successful in selective immunoabsorption of a single immunotype of antibody from a mixture of Pseudomonas antibodies. In addition, we have been successful in eluting the antibody, and it is active by passive hemagglutination assays. Bactericidal assays are currently being conducted.

Clinical and laboratory studies of anaerobic infections in trauma patients continue under the direction of W. A. Altemeier. These include infections by the Peptostreptococcus, Bacteroides, and Clostridia.

Quantitative Aspects of Pseudomonas aeruginosa Infection in Burned Mice. D. D. Stieritz and I. A. Holder. (Abstract)

To further elucidate the role of Pseudomonas aeruginosa in burn wound sepsis, mice were subjected to a non-lethal 10-second flame burn (approximately 30% total body surface), followed immediately by subcutaneous injection of various organisms into the burn site. The LD_{100} value for Ps. aeruginosa was 10^1 as opposed to an LD_{50} value in 10^6 in normal animals. Injection of Ps. aeruginosa by other routes or into unburned skin gave higher values. The LD_{50} values for Klebsiella sp., Escherichia coli, and Candida albicans were 10^5 in the burned animal. Bacterial counts following injection into the burn site showed rapid proliferation in the burn skin, approaching 10^8 organisms per gram of tissue, whereas counts in liver, spleen, kidney, lung and blood were detectable only after 20 hours, with a mean time to death of 32 hours. Administration of gentamicin at 24 hours post-injection significantly reduced the numbers of organisms in the spleen and liver (versus untreated-controls), without affording increased survival. While other investigators have demonstrated increased susceptibility of burned animals to Ps. aeruginosa, studies with our animal model would suggest that this degree of increased susceptibility is peculiar to this organism.

d. Incidence of Non-Sporulating Anaerobes in Surgical Infections.

Cultures of non-sporulating anaerobes recovered from surgical infections and maintained as frozen stock cultures over the past 14 years were re-identified by current methods for classification. Of 826 specimens (562 patients), 689 (83%) yielded bacterial growth with 403 (58.8%) of the positive cultures containing anaerobic bacteria. (Table 1) Nearly 12 per cent of the positive specimens contained only anaerobes. The average number of anaerobic species per specimen was 2.9 and ranged from one to seven.

7.3 d. Continued

Table 1. Totals of Cultures Between 1960 and 1975, excluding Stool, Sputum, Urine and Blood Specimens.

Unit	Number	% of Total	% of Positive
Patients	562		
Total specimens cultured	826	100.0	
Specimens positive by culture	689	83.4	100.0
Specimens yielding facultative anaerobes	608	73.0	88.2
Specimens yielding anaerobes	403	48.8	58.5
Mixed, facultative and anaerobes	322	39.0	46.7
Facultative anaerobes only	286	34.6	41.5
Anaerobes only	81	9.8	11.8
Negative by culture	137	16.6	

Table #2 shows that 57 per cent of 370 randomly selected anaerobe-containing specimens yielded at least one anaerobic gram-negative rod and that 20 per cent of these specimens contained these organisms as the only anaerobe present. The need for proper, rapid diagnosis was evident in the fact that 40.5 per cent of the specimens yielded penicillin-resistant Bacteroides fragilis strains. The per cent of clinical specimens containing the various types of anaerobes is shown in Table #2.

Table 2. Types of Bacteria found in 370 Anaerobe-Positive Specimens from 261 Patients.

Anaerobe	% of Specimens with at least one
Gram-negative rod	57%
Gram-positive rod (less Clostridium)	40%
Gram-positive cocci	45%
Clostridium	13.5%
Bacteroides fragilis	40.5%

7.3 Continued:

A complete breakdown of the anaerobes identified is given in Table #3.

Table 3. Listing of Anaerobes from 370 Specimens (261 Pts.)

Organism	Number
Bacteroides fragilis ssp. fragilis	80
B. fragilis ssp. thetaiotamicron	43
B. fragilis ssp. distasonis	16
B. fragilis ssp. vulgatus	9
B. fragilis ssp. ovatus	1
B. fragilis (other)	25
B. species	43
B. melaninogenicus ssp. asaccharolyticus	32
B. melaninogenicus ssp. intermedius	3
B. melaninogenicus ssp. melaninogenicus	2
B. putredinis	7
B. coagulans	4
B. corrodens	4
B. oralis	1
B. praeacutus	1
B. biacutus	1
B. ruminacola ssp. brevis	1
Fusobacterium nucleatum	8
F. species	6
F. naviforme	1
F. aquatile	2
F. varium	1
F. gonidiaformans	1
F. symbiosum	1
F. russii	1
F. mortiferum	1
Eubacterium lentum	21
Eub. cylindroides	5
Eub. limosum	5
Eub. alactolyticum	2
Eub. species	2
Bifidobacterium species	4
B. longum ssp. longum	2
B. infantis (other)	3
B. adolescentis variety B	1
B. adolescentis variety D	1
B. bifidum	1
B. breve	1

7.3 Continued

Table #3. Listing of Anaerobes from 370 Specimens (261 Pts.) (Continued)

Organism	Number
Lactobacillus species	5
L. bulgaricus	1
L. casei variety casei	1
L. buchneri	1
Propionibacterium acnes	87
P. avidum	6
P. lymphophilum	6
P. granulosum	2
P. species	7
Peptococcus magnus	75
Pc. asaccharolyticus	32
Pc. constellatus	6
Pc. prevotii	31
Pc. variabilis	9
Pc. morbillorum	2
Peptostreptococcus anaerobius	30
Ps. intermedius	22
Ps. micros	14
Ps. productus	4
Ps. parvulus	1
Veillonella parvula	10
Veillonella species	1
Acidaminococcus fermentans	1
Megasphaera elsdenii	1
Clostridium perfringens	24
Cl. innocuum	7
Cl. species	7
Cl. ramosum	4
Cl. cadaveris	2
Cl. butyricum	3
Cl. tertium	2
Cl. sporogenes	2
Cl. septicum	2
Cl. pasteurianum	2
Cl. tyrobutyricum	1
Cl. sartagoformum	2
Cl. bifermentans	1
Unidentified gram-positive cocci	26
Unidentified gram-variable cocci	2
Unidentified anaerobe.	8

7.3 Continued:

d. Continued

Studies on the Role of Bacteroidaceae and L-forms in Thromboembolic Disease

Studies on the mechanism(s) of acceleration of blood coagulation by lipopolysaccharide from Bacteroides sp. and Fusobacterium mortiferum have been extended the past year. This work was reported at the National Meeting of the American Society for Microbiology, submitted to the University of Cincinnati by H. S. Bjornson as a dissertation for the Ph.D. degree, and a manuscript has been published in Infection and Immunity.

Due to the high incidence of thromboembolic disease and infection with non-sporulating gram-negative anaerobes or organisms of pleomorphic "L-ty" morphology, the effects of Bacteroides sp. and the bacterial and L-forms of F. mortiferum on blood coagulation in vivo and in vitro were determined. The methods for demonstrating in vivo acceleration of coagulation were the capillary microclotting time technic in mice and Lee White clotting time in rabbits. The method for demonstrating in vitro acceleration of coagulation was the recalcified clotting time of platelet poor human or rabbit plasma in the presence of a platelet phospholipid substitute, chloroform extract of brain.

The anaerobic microorganisms accelerated blood coagulation by a mechanism which did not require platelets. In addition, the LPS of the microorganisms had no effect on non primate platelets in vivo or human platelets in vitro. The clot-promoting activity appeared to be dependent upon the lipid A moiety of the LPS of the anaerobes. The mechanism by which the LPS and lipid A of the anaerobic microorganism accelerated coagulation was by the activation of factors XI and/or XII, the initial components of the intrinsic pathway of coagulation.

Lipid A, but not the LPS, of E. coli was also found to accelerate coagulation in vivo and in vitro by a platelet independent mechanism. In addition, Salmonella minnesota R595 a mutant whose cell wall LPS contains only lipid A and KDO also accelerated coagulation by a platelet independent mechanism. These observations lend support to the hypothesis that appropriately exposed lipid A, irrespective of the source may be capable of accelerating coagulation.

The similarity in the coagulation effects of lipid A of E. coli and the gram-negative anaerobic microorganisms may be related to their biochemical similarity. The lipid A of the anaerobes was demonstrated to be qualitatively similar in fatty acid content and identical in antigenic determinants to the lipid A of E. coli.

7.3 Continued:

d.

Humoral factors present in human and rabbit plasma were demonstrated to be capable of neutralizing the clot-promoting effects of LPS and lipid A of members of the family Bacteroidaceae. Plasma obtained from rabbits immunized with the bacterial form of F. mortiferum neutralized the clot-promoting effects not only of homologous LPS and lipid A, but also the LPS and lipid A of Bacteroides sp. and the lipid A of E. coli. Plasma obtained from rabbits immunized with the L-form of lipid A of F. mortiferum had no neutralizing capacity. Plasma samples from patients convalescing from bacteremia due to members of the Bacteroidaceae of bacterial morphology were also shown to neutralize the clot promoting effects of lipid A. However, plasma samples obtained from patients with recurrent thromboembolic disease, with blood cultures positive for pleomorphic organisms of "L-type" morphology, were unable to neutralize the clot-promoting effects of lipid A. Preliminary evidence suggests that the humoral factors present in immune rabbit plasma, responsible for neutralizing the clot-promoting effects of lipid A and LPS of the anaerobes, are immunoglobulins of the LgG class. These observations may provide a basis for the development of immunotherapy for the treatment of patients with recurrent thromboembolic disease.

Development and Application of an Indirect Fluorescent Antibody Procedure for Identification of the Family Bacteroidaceae

Antisera have been prepared against the five subspecies of Bacteroides fragilis, B. melaninogenicus ssp., asaccharolyticus and intermedius, Fusobacterium mortiferum, F. nucleatum, F. symbiosum, F. varium, and Fusobacterium species. Continuing studies of in vitro specificity involving the reactions of 31 antisera with 40 homologous and heterologous bacterial cell antigens of the Bacteroidaceae have substantiated our previous reports of specificity. All reactions have been at least species specific with a significant degree of group or strain specificity occurring within species of Fusobacterium. Subspecies specificity with the existence of antigenic groups with various subspecies was characteristic of Bacteroides fragilis. Antisera demonstrated occasional fluorescence with strains of Escherichia, Klebsiella, Streptococcus, Staphylococcus, and Propionibacterium. Adsorption of the involved antisera and normal serum with appropriate bacterial cells eliminated this unwanted fluorescence without altering the effectiveness of this procedure. Because of the high degree of specificity, polyvalent antisera pools were employed to facilitate screening of direct smears.

7.3 Continued:

d. Continued

As reported in the previous annual report, this procedure has been applied to 30 clinical specimens from liver, brain, perirectal, and renal abscesses, cellulitis, and various other aspirate and exudates. F.A. results obtained with direct smears correlated well with culture results and this procedure continues to be promising for rapid diagnosis of infections caused by these organisms. All positive F.A. reactions obtained with direct smears have been verified by similar F.A. reactions obtained with pure cultures of the organisms isolated from clinical specimens.

These data was presented at the National Meeting of the American Society for Microbiology, May, 1975.

Studies on Significance of Non-sporulating Anaerobes in Surgical Infections and Mechanisms of Pathogenicity.

The experimental liver abscess model described earlier continues to serve as an excellent model for studies on mechanisms of pathogenicity of Fusobacterium necrophorum. This work also was presented at the National Meeting of the American Society for Microbiology.

Organ Specificity of Fusobacterium necrophorum in Experimental Infections in Mice. (Abstract)

P. M. Abe, E. S. Lennard, J. W. Holland, and E. O. Hill

Intraperitoneal inoculation of mice with a bovine strain of Fusobacterium necrophorum results in development of liver abscesses. The correlation between this experimental model and liver abscesses in humans led to studies of the histopathology and pathogenesis of these infections. At hourly and daily intervals bacterial counts were made of lung, liver, and spleen, and histologic sections were stained with H & E and indirect fluorescent antibody technic. Though bacterial counts were 10^8 - 10^9 cells/gram in liver and spleen for 17 days, abscesses developed only in the liver. In immunized mice, bacterial uptake in these organs was transient and cleared by 6 hours. Predisposition to abscess formation in the liver suggests inability of macrophages to cope with F. necrophorum.

7.3 Continued

d. Clinical Application of an Indirect Fluorescent Antibody Technic for the Bacteroidaceae (Abstract)

L. R. Stauffer, J. W. Holland, and E. O. Hill

An indirect F.A. procedure, evaluated by staining direct smears of specimens from experimental animal infections and human infections, appears to be a specific and practical method for rapid, presumptive diagnosis of infections involving the Bacteroidaceae. Antisera, prepared in rabbits against the Bacteroides and Fusobacterium species commonly found in clinical material, were used with commercially available fluorescein isothiocyanate labeled anti-globulin globulin. Results of F.A. applied to direct smears of specimens correlated well with culture results and indicated a high degree of species specificity. To date, 25 clinical specimens have been tested. Of 11 specimens yielding Bacteroidaceae, 9 were presumptively diagnosed by F.A. and confirmed by culture to contain subspecies of B. fragilis and/or Fusobacterium species. Use of pooled polyvalent antisera facilitates application of this method.

e. Septic Shock Studies

Objective:

Septic shock continues to be a serious problem in clinical surgery. The objective of this study is to improve the treatment of septic shock and reduce its mortality, morbidity, and sequelae occurring as complications of post-trauma, postoperative, and other surgical infections.

Background:

Life-threatening shock-like states occurring in patients with serious sepsis continues to be one of the most important and unsolved problems in clinical surgery.

It may occur as a complication of sepsis in the post-trauma or postoperative patient. The principal investigator was commissioned to go to the battle area during the Korean War by the Surgeon-General to study the cause of delayed shock which was developing in a large number of casualties two to five days after injury. His studies showed a causal relationship of infection to the delayed shock in many of these patients.

Septic shock continues to be a serious problem in surgical practice today, particularly after such injuries as penetrating wounds of the abdomen, compound fractures, extensive lacerations, and gunshot wounds. Peritonitis, infected burns, intraabdominal abscesses, retroperitoneal abscesses, crepitant cellulitis of the abdominal wall, gas gangrene, and septicemia are examples of trauma and postoperative infections of this type.

7.3 e. Continued:

More recently nosocomial infections have become recognized as another prevalent source of septic shock. Examples of such nosocomial infections include those related to invasive diagnostic and therapeutic procedures of the urinary tract, continuous intravenous infusion or parenteral feeding, post-tracheostomy infections of the respiratory tract, and antibiotic related or secondary infections.

Our studies have shown that many microorganisms have been identified as etiologic agents in septic shock. These have been shown to be aerobic or anaerobic and both gram-positive and gram-negative. They include hemolytic Staphylococcus aureus, beta hemolytic Streptococcus, the Clostridia, Bacteroides, Proteus, Escherichia coli, Pseudomonas, Aerobacter-Klebsiella, Serratia, and others.

Our investigations previously reported under MD-2531 have shown that serious infections with life-threatening septic shock have not been significantly reduced by antibiotic therapy after more than a third of a century of its general use.

During the past three years, an investigative program has been developed in experimental animals (dogs, rabbits, and mice) to explore in depth the nature of septic shock for the purpose of acquiring more information concerning the localization of selected bacterial toxins and their pathophysiologic effects which might be useful in the understanding and management of patients with sepsis.

Based upon the types of clinical infections associated with sepsis and septic shock which we studied at the Cincinnati General Hospital, three bacterial toxins were selected, all of which were different in origin and composition. These toxins were as follows:

1) Endotoxin of E. coli -- a polysaccharide

2) The alpha exotoxin of hemolytic Staphylococcus aureus - a protein enzyme

3) Alpha or lethal exotoxin of Cl. perfringens - lecithinase

Using dogs, rabbits, and mice, the pathophysiologic effects following the intravenous injection of each of these toxins into separate animals were carefully determined, measured, and recorded. The results have been interesting and will be important in providing a better understanding of the nature and control of septic shock.

Research Plan:

The parameters measured included systolic arterial pressure, arterial pulse pressure, portal venous and central venous

7.3 e. Continued

pressures, peripheral vascular resistance, pulmonary arterial pressure, cardiac output, hematologic changes, alterations in blood chemistry levels, and pathological changes in the lung, liver, and other organs affected by the toxins.

Protocol.

The protocol used since June 20, 1974, in a series of dogs consisted of the following. Thirteen to eighteen Kg. dogs were anesthetized with 65-70 mg. of Nembutal and 1 cc. Innovar. An endotracheal tube was inserted in each and secured in place. The left groin was then prepped with phisohex, painted with tincture merthiolate, and draped in an appropriate sterile fashion. A 5 cm. incision was made in the medial aspect of the upper thigh and developed in layers down to the femoral artery. Bleeding was controlled by direct pressure and/or suture. The femoral artery was cannulated and secured in place by proximal and distal ligatures of 4-0 black silk. The cannula was then flushed with heparinized saline (1 cc. heparin diluted in 500 cc. normal saline)and connected to a mercury manometer for measurement of arterial blood pressure. The wound was closed in layers using 4-0 black silk sutures.

Catheters were also placed for mean pressure, pulmonary arterial pressure, and central venous pressures. In the later experiments cardiac output was measured by the thermodilution method. The cannula was brought out through the incision and the wound covered with a dry sterile dressing. An intravenous line was then inserted into the right foreleg. Measured amounts of normal saline solution were infused slowly throughout the procedure.

Normal baseline levels of respirations, heart rate and arterial blood pressures were obtained. At 2:57 p.m., the E. coli endotoxin (2.0 mg./Kg) was injected through the IV line in one bolus. Blood pressures, levels, respirations, and heart rates, urinary output were then monitored for the next 105 minutes at somewhat regular intervals. Blood samples were also drawn for hemoglobin studies, and HCT, CBC, Platelets, CO_2, PCO_2 and PO_2 at regular intervals. Lung and liver biopsies were obtained after 105 minutes, and the dog was sacrificed.

Results:

During the past year septic shock studies were conducted using a dose of 2 mgm/Kg of E. coli endotoxin in dogs. A typical biphasic systemic arterial curve was obtained with a precipitous primary fall in arterial pressure, a period of pressure recovery, and a secondary fall ending in death in 300 minutes. There was an elevation in portal venous flow associated with the primary systemic arterial hypotension, and a marked and sustained decrease in cardiac output. During the primary arterial hypotensive period,

7.3 e. Continued

there was a marked increase in peripheral resistance. Significant pulmonary, cardiac, hepatic, and renal pathological changes occurred.

During the rise in systemic arterial pressure in the early part of the secondary phase, the mean pulmonary arterial usually rose temporarily and then gradually fell until the time of death.

Similar changes were obtained in dogs with I.V. injections of alpha toxin (exotoxin) of the hemolytic Staphylococcus aureus and the alpha toxin (exotoxin) of the Clostridium perfringens.

Using this protocol a total of 27 dogs injected with E. coli endotoxin have been studied. Figure I shows the typical reactive hemodynamic levels which were recorded in these dogs after the I.V. E. coli endotoxin injection. Of particular interest to us were the following:

1. The prompt and precipitous primary fall (post injection) in the mean systemic arterial pressure after a temporary and transient rise.

2. A period of recovery with a partial and temporary rise of the mean systemic arterial pressure to a level of usually 60-75% of that measure pre-injection.

3. A secondary and progressive fall in the mean systemic arterial pressure over a period of 100-120 minutes until the death of the animal.

4. As the primary fall in arterial pressure occurred, the portal venous pressure rose sharply and then fell as the arterial pressure recovered.

5. Of great interest also was the sudden and precipitous fall in cardiac output which remained depressed throughout the duration of the experiments until the animal's death.

6. Also of great interest was the sudden rise in peripheral resistance simultaneously with the primary mean system arterial fall and the rise in portal venous pressure. The peripheral resistance then gradually and progressively fell over the next 50 minutes.

7. Biopsies of the lung at the end of 120 minutes showed extensive hemorrhagic infiltration of the interalveolar septa, atelectasis, induration, and decreased pulmonary compliance.

Blood Studies.

The summary of laboratory data obtained in this group of animals is illustrated in the following table. The figures are averaged.

7.3 e. Continued

SEPTIC SHOCK STUDIES

Laboratory Data
(Average Values)
1974--75

	BASELINE	60 MINUTES
Red Blood Count	5,682,000	5,643,000
Hemoglobin	13.7	14.
White Blood Count	4,828	3,716
Platelets	188,100	56,160
Total Protein	5.6	4.47
Albumin	2.54	2.15
Glucose	149	190
Blood Urea Nitrogen	19	20
Creatinine	0.74	1.1
SGOT	29	38
LDH	94	243.
Total Bilirubin	0.12	0.15
Alkaline Phosphates	34	33
Uric Acid	0.4	2.55
Na^+	148	147
K^+	3.7	7.3
Cl^-	114	114.7
Ca^{++}	9.42	8.65

7.3 e. Continued

SEPTIC SHOCK STUDIES

Laboratory Da
(Average Values)
1974--75

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Total Bilirubin	0.12	0.15
Alkaline Phosphates	34	33
Uric Acid	0.4	2.55
Na^+	148	147
K^+	3.7	7.3
Cl^-	114	114.7
Ca^{++}	9.42	8.65

7.3 e. Continued

Figure I.

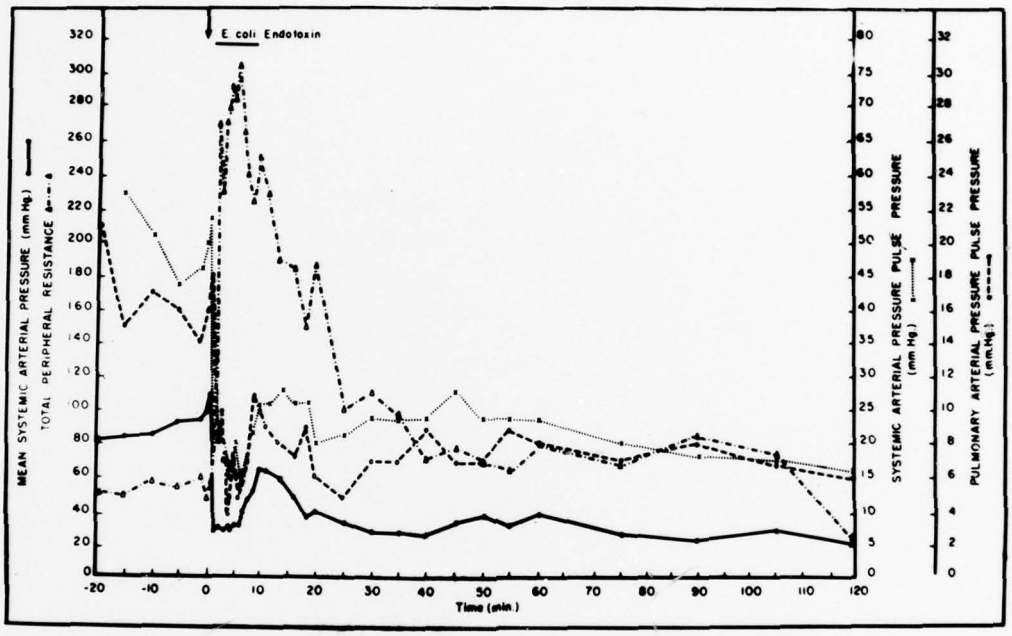

Figure I. Chart showing hemodynamic changes following intravenous injection of 2.0 mg./Kg of E. coli endotoxin, in an adult dog.

7.3 e. Continued

Significance of these Studies in Relation to Human Health

Sepsis and septic shock still present significant problems in clinical practice, the latter carrying a high mortality ranging from 40% to 60% or more, depending upon the type of septic focus, the nature of the infecting microorganism, and the type of treatment.

Septic shock is not a single clinical entity. Rather, it is a syndrome in which a failure of the systemic arterial and visceral arterial tissue perfusion are important integral aspects. Cardiac, pulmonary, renal, and other visceral failures may follow as part of the irreversibility phase. The tissue hypoperfusion deficit is at first reversible and only secondarily does it become irreversible.

Our data were highly informative concerning the nature of septic shock and suggestive that an important advance in our therapy of this serious and highly fatal type of shock could be made by the intravenous infusion of a drug which would increase cardiac output, decrease peripheral resistance, and maintain a controlled mean arterial pressure which would minimize secondary visceral damage, prevent irreversibility, and reduce mortality and morbidity.

We are proceeding with investigations in this direction.

It is understood, of course, that timed surgical intervention as indicated, appropriate antibiotic therapy, and general supportive therapy would be used as needed for the treatment of septic shock in clinical practice as needed.

7.3 Continued:

f. Studies in Staphylococci Resistance to Penicillin and Other Antibiotics; Cyclic Variations

Studies in the cyclic variation of the resistance of Staphylococcus aureus to antibiotics has continued and has been reported at the meeting of the American Surgical Association on May 1, 1974. The per cent frequency of resistance to various antibiotics on a quarterly basis from January 1, 1973 - March 30, 1974 can be seen in the following tables. Beginning with January 1, 1974 the battery of antibiotics has been updated. Bacitracin and novobiocin were dropped. Two of the newer tetracycline analogues (doxycycline and minocycline) and clindamycin have been added. It is of interest to note that during the first quarter of 1974 none of the Staph. aureus tested were resistant to minocycline whereas 4.3% were resistant to tetracycline.

There continues to be a difference in resistance when comparing the Kirby Bauer and two disc methods of testing. The most striking example of this is seen with penicillin. For the first quarter of 1974, 83.6% of the isolates tested were resistant using the Kirby Bauer method as compared with 4.3% using the two disc method.

Phage typing has continued on all isolates from patients with infections seen and studied at the Cincinnati General and Holmes Hospitals.

Quarterly variations in Resistance os Staphylococcus aureus to Penicillin, Chloramphenicol, and Tetracycline.

Year	Quarter	No. of Strains Tested	Penicillin % Resistant		Tetracycline % Resistant		Chloramphenicol % Resistant	
			Kirby-Bauer	2-Disc Method	Kirby-Bauer	2-Disc Method	Kirby-Bauer	2-Disc Method
1972	1	92	84	2	32	22	0	0
	2	51	80	4	47	47	0	0
	3	189	88.4	0	10.1	6.9	0	0
	4	348	79.3	0.9	7.2	5.2	0	0
1973	1	481	87.5	0.6	13.5	10.6	0.2	0
	2	611	85.1	4.7	7.7	5.1	0	0
	3	153	91.5	9.2	7.8	3.3	0	0
	4	73	69.9	6.8	13.7	4.1	0	0
1974	1	439	83.6	4.3	4.3	0.9	0	0

PER CENT FREQUENCY OF RESISTANCE OF STAPHYLOCOCCUS AUREUS TO ANTIBIOTICS

January 1 - March 31, 1974

Source	Total Tested		Penicillin	Bacitracin	Chloramphenicol	Erythromycin	Novobiocin	Tetracycline	Neomycin	Ampicillin	Methicillin	Prostaphlin
Pus	116	%	89.7	12.9	0.0	19.8	0.0	22.4	14.7	89.7	0.0	0.0
Non-purulent Infections	102	%	89.2	1.0	0.0	8.8	0.0	11.8	8.8	89.2	0.0	0.0
Nose, throat and sputum	215	%	87.0	2.3	0.0	10.7	0.5	12.6	8.8	86.5	0.0	0.0
Blood	7	%	42.9	0.0	0.0	0.0	0.0	0.0	0.0	42.9	0.0	0.0
Stool	26	%	96.2	0.0	0.0	0.0	0.0	0.0	0.0	96.2	0.0	0.0
Urine	7	%	71.4	0.0	0.0	0.0	0.0	0.0	0.0	71.4	0.0	0.0
Other	8	%	75.0	0.0	0.0	0.0	0.0	0.0	0.0	75.0	0.0	0.0

January 1 - March 31, 1974

Source	Total Tested		Gentamicin	Kanamycin	Keflin	Lincocin	Nafcillin	Streptomycin	Doxycycline
Pus	116	%	0.0	14.7	0.0	0.0	0.0	19.8	22.4
Non-purulent Infections	102	%	0.0	8.8	0.0	0.0	0.0	7.8	7.8
Nose, throat and sputum	215	%	0.0	8.8	0.0	0.0	0.0	11.2	7.4
Blood	7	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stool	26	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Urine	7	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other	8	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0

PER CENT FREQUENCY OF RESISTANCE OF STAPHYLOCOCCUS AUREUS TO ANTIBIOTICS

April 1 - June 30, 1974

Source	Total Tested										
Pus	193	% 83.9	3.1	0.0	7.2	0.51	7.8	4.7	84.4	0.0	0.0
Non-purulent Infections	68	% 91.2	0.0	0.0	1.5	0.0	5.9	2.9	91.2	0.0	0.0
Nose, throat, and sputum	278	% 86.3	0.71	0.0	3.9	0.71	7.2	2.1	84.9	0.0	0.0
Blood	8	%100.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
Stool	22	% 77.3	0.0	0.0	13.6	0.0	9.1	0.0	77.3	0.0	0.0
Urine	16	% 50.0	6.3	0.0	18.7	0.0	18.7	0.0	43.7	0.0	0.0
Other	25	% 92.0	8.0	0.0	12.0	0.0	12.0	4.0	92.0	0.0	0.0

April - June 30, 1974

Source	Total Tested		Gentamicin	Kanamycin	Keflin	Lincocin	Nafcillin	Streptomycin	Doxycycline
Pus	193	%	0.0	3.6	0.0	0.0	0.0	5.7	5.7
Non-purulent Infections	68	%	0.0	2.9	0.0	0.0	0.0	2.9	1.5
Nose, throat and sputum	278	%	0.0	1.4	0.35	0.0	0.35	4.7	5.4
Blood	8	%	0.0	0.0	0.0	0.0	0.0	12.5	0.0
Stool	22	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Urine	16	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other	25	%	0.0	4.0	0.0	0.0	0.0	16.0	12.0

PER CENT FREQUENCY OF RESISTANCE OF STAPHYLOCOCCUS AUREUS TO ANTIBIOTICS

July 1 - September 30, 1974

Source	Total Tested		Gentamicin	Kanamycin	Keflin	Lincicin	Nafcillin	Streptomycin	Doxycycline
Pus	89	%	0.0	0.0	0.0	0.0	0.0	3.4	4.5
Non-purulent Infections	20	%	0.0	0.0	0.0	0.0	0.0	10.0	15.0
Nose, throat and sputum	30	%	0.0	0.0	0.0	0.0	0.0	6.7	10.0
Blood	3	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stool	2	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Urine	4	%	0.0	0.0	0.0	0.0	0.0	0.0	50.0
Other	5	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0

PER CENT FREQUENCY OF RESISTANCE OF STAPHYLOCOCCUS AUREUS TO ANTIBIOTICS

July 1 - September 30, 1974

Source	Total Tested		Penicillin	Bacitracin	Chloramphenicol	Erythromycin	Novobiocin	Tetracycline	Neomycin	Ampicillin	Methicillin	Prostaphlin
Pus	89	%	88.8	0.0	0.0	7.9	1.1	4.5	0.0	91.0	0.0	0.0
Non-purulent Infections	20	%	90.0	0.0	0.0	10.0	0.0	15.0	0.0	90.0	0.0	0.0
Nose, throat and sputum	30	%	90.0	0.0	0.0	10.0	0.0	10.0	0.0	90.0	0.0	0.0
Blood	3	%	100.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
Stool	2	%	100.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
Urine	4	%	100.0	0.0	0.0	25.0	0.0	50.0	0.0	100.0	0.0	0.0
Other	5	%	100.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0

PER CENT FREQUENCY OF RESISTANCE OF STAPHYLOCOCCUS AUREUS TO ANTIBIOTICS

October 1 - December 31, 1974

Source	Total Tested		Penicillin	Bacitracin	Chloramphenicol	Erythromycin	Novobiocin	Tetracycline	Neomycin	Ampicillin	Methicillin	Prostaphlin
Pus	41	%	73.2	4.9	0.0	2.4	0.0	7.3	0.0	68.3	0.0	0.0
Non-purulent infections	2	%	50.0	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0
Nose, throat and sputum	14	%	92.9	0.0	0.0	14.3	0.0	35.7	0.0	85.7	0.0	0.0
Blood	2	%	0.0	0.0	0.0	50.0	0.0	50.0	0.0	0.0	0.0	0.0
Stool	4	%	75.0	0.0	0.0	0.0	0.0	0.0	0.0	75.0	0.0	0.0
Urine	4	%	50.0	0.0	0.0	0.0	0.0	25.0	0.0	50.0	0.0	0.0
Other	6	%	33.3	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0

October 1 - December 31, 1974

Source	Total Tested		Gentamicin	Kanamycin	Keflin	Lincocin	Nafcillin	Streptomycin	Doxycycline
Pus	41	%	0.0	0.0	0.0	0.0	0.0	7.3	4.9
Non-purulent Infections	2	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nose, throat and sputum	14	%	0.0	0.0	0.0	0.0	0.0	42.9	21.4
Blood	2	%	0.0	0.0	0.0	0.0	0.0	50.0	50.0
Stool	4	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Urine	4	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other									

PER CENT FREQUENCY OF RESISTANCE OF STAPHYLOCOCCUS AUREUS TO ANTIBIOTICS

January 1 - March 31, 1975

Source	Total Tested		Penicillin	Chloramphenicol	Erythromycin	Minocycline	Tetracycline	Neomycin	Ampicillin	Methicillin	Gentamicin	Kanamycin	Keflin
Pus	200	%	83.0	0.0	3.0	0.0	5.0	0.0	83.0	0.0	0.0	0.5	0.0
Non-purulent Infections	69	%	87.0	0.0	2.9	0.0	5.8	0.0	87.0	0.0	0.0	0.0	0.0
Nose, throat, and sputum	103	%	78.6	0.0	1.0	0.0	3.9	0.0	78.6	0.0	0.0	0.0	0.0
Blood	18	%	100.0	0.0	5.6	0.0	5.6	0.0	100.0	0.0	0.0	0.0	0.0
Stool	16	%	93.8	0.0	6.2	0.0	0.0	0.0	87.5	0.0	0.0	0.0	0.0
Urine	10	%	90.0	0.0	0.0	0.0	0.0	0.0	90.0	0.0	0.0	0.0	0.0
Other	22	%	86.4	0.0	4.5	0.0	4.5	0.0	86.4	0.0	0.0	0.0	0.0

January 1 - March 31, 1975

Source	Total Tested		Lincomycin	Clindamycin	Doxycycline	Streptomycin
Pus	200	%	0.0	0.0	2.5	2.0
Non-purulent	69	%	0.0	0.0	4.3	1.4
Nose, throat and sputum	103	%	0.0	0.0	0.0	0.0
Blood	18	%	0.0	0.0	5.6	5.6
Stool	16	%	6.2	6.2	0.0	0.0
Urine	10	%	0.0	0.0	0.0	0.0
Other	22	%	0.0	0.0	4.5	0.0

7.4 & 7.5
To Evaluate Further the Effectiveness of Pseudomonas vaccine and Pseudomonas hyperimmune globulin in the prevention and control of Pseudomonas infections in burned and other seriously injured patients, and

To Investigate in Burn Patients the Relationship between antibody formation developing from immunization with Pseudomonas vaccine and restoration of opsonic activity for Pseudomonas aeruginosa.

Specific Aims

The specific aims of this project are twofold: first to continue evaluation of a polyvalent pseudomonas antigen (Pseufogen, Parke-Davis) and its corresponding immune globulin in the prevention and treatment of Pseudomonas infection in seriously burned individuals, and second to separate and purify natural and immune Pseudomonas antibodies and study differences in biological effect in vitro and in vivo.

Background Information and Hypothesis

It is known that specific antibodies synthesized at different times throughout an immunization schedule possess different affinities for the complementary antigen, presumably from small variances in the structure of the Fab portions of the molecules. One or more of the sub-populations might comprise the group of antibody referred to as natural antibody, the titers of which are usually low. The origin of natural antibody is controversial, but we have shown that natural antibody for Pseudomonas is not present in germfree rats whereas it is present in conventional rats. When germfree rats are fed live Pseudomonas aeruginosa they soon develop hemagglutinating antibodies in their circulation. This suggests that natural antibody arises from oral immunization in response to a specific antigen. We have previously shown that the antibody in normal human gammaglobulin (natural antibody) activates the complement system in a way which is different from immune antibody raised by an active parenteral immunization with a specific antigen. One portion of our investigations has been to try to separate mixtures of natural and immune antibody to perform precise analysis of the physical and chemical differences between the two types of IgG reacting with a single antigen. The use of affinity chromatography for the isolation and separation of specific proteins and other biologically active molecules from complex mixtures has been employed for several years. It appears to be a perfect tool for these studies. Our hypothesis is that natural antibody and immune antibody to Pseudomonas aeruginosa activate the complement sequence in a different manner and affect host resistance in a different manner because of differences in affinity to a specific antigen on the cell wall of Pseudomonas aeruginosa.

7.4 & 7.5 Continued:

We feel that high affinity immune antibody will be far superior for the treatment of active infections than will an equal amount of biologically active natural antibody.

Objectives

Our primary objective at the present time is to separate natural and immune antibodies to Ps. aeruginosa, demonstrate that they have differences in affinity which relate to their biochemical structure, and test their relative biological potencies both in vitro and in vivo.

Studies Conducted During the Past Year

In the report for the first six months work during this year, there was an error in the number of patients that we indicated had been vaccinated with pseudomonas heptavalent antigen (Pseudogen). The report should have read 444 patients rather than 515. At any rate, in the last group of 308 consecutive patients, mortality from infections from Pseudomonas aeruginosa had been eliminated. There has been one death from an infection caused by Pseudomonas aeruginosa since that last report.

It has recently been necessary to change the protocol to vaccinate only those patients with 40% or more total body surface area rather than 20% because of a limited availability of the antigen.

The studies on separation of natural and immune antibodies during the past year have been significantly curtailed because of a lack of funding. However, we have been able to show by affinity chromatography that we can elute specific immune antibody for Pseudomonas aeruginosa immunotype 4 with different elution and absorption peaks than specific natural antibody against Pseudomonas aeruginosa immunotype 4. The tests of functional activity have not yet been performed.

Proposed Studies for the Coming Year

We propose to clearly define the difference between natural and immune antibody based upon differences in affinity for the specific antigen, by biochemical analysis of the two types of specific immune antibody, and by analysis of the functional differences in its role in immune reactions.

Research Plan

During the past year, preliminary studies, which have been funded from internal sources, have been done to design a technic for immune extraction of specific antibody with columns containing a support linked covalently to the corresponding antigen. These studies have been performed in collaboration with Dr. James Ogle of the Department of Biochemistry, University of Cincinnati.

7.4 & 7.5 Continued

Specific antibody for Pseudomonas aeruginosa immunotype 4 has been extracted from mixtures of gammaglobulin with columns of high capacity which will provide yields of 70% to 90% with a single pass through the column. By producing elution gradients and loading the columns with either normal gammaglobulin containing anti-type 4 antibody or immune human gammaglobulin containing type 4 antibody, we have been able to demonstrate differences in absorption to the column and different patterns of elution with different types of gradients. A large number of samples have now been collected, and we now wish to study these for biochemical and functional differences.

Studies of opsonic support will be made using best inactivated sera, specific antibody depleted sera, serum free tests, and serum free tests with specific components of the classical and alternate pathway of complement added individually and in combination. This type of testing will be repeated for each of the seven immunotypes of Pseudomonas aeruginosa providing the basic antigen for "Pseudogen." After the identification of specific functional differences in homogenous antibodies separated by affinity technics, an attempt will be made to characterize the differences in immunoglobulin structure on a biochemical basis.

The Significance of Work Proposed

At the present time, it would appear that immune anti-Pseudomonas antibody does not require participation of the alternate pathway of complement, but natural antibody does. We have recently shown that there is a consumption of alternate pathway components in severe thermal injury complicated by infection, resulting in a severe increase in susceptibility to infection by both the infecting and the other pathogens. It is probable that immune globulin will be significantly more potent biologically than antigen-specific natural antibody. This would have profound importance in the treatment of specific bacterial infections since it would be possible to administer relatively small amounts of highly effective antibody which has been fractioned by affinity chromatographic procedures. Infection,with problem pathogens such as Pseudomonas aeruginosa, is one of the leading causes of death following traumatic injury.

Contract No. DAMD-17-75-C-5018

FINAL REPORT

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